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## Research Note

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A SIMPLE METHOD FOR DETECTING INCOMPATIBILITY  
IN 2-YEAR-OLD GRAFTS OF DOUGLAS-FIR

by

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Introduction

Graft incompatibility has become one of the more important problems facing Douglas-fir orchardists. It has been 10 years since large-scale grafting was started in Oregon and Washington Douglas-fir seed orchards. Some mortality from graft incompatibility began to appear as early as the fall of the year of grafting. Since that time, more losses have occurred each year in the early incompatible clones (E.I.). An even more disturbing loss has resulted from delayed incompatibility (D.I.). Clones with D.I. showed good early survival and no external symptoms of incompatibility but in later years developed a scion overgrowth symptom--such as first described by Duffield and Wheat.<sup>1/</sup> Finally, many overgrown grafts died, and many more are still dying. The seriousness of the problem is indicated by a recent inventory, now in our files, made by three seed orchard operators. The survey of 42 clones revealed that 46 percent of the 3,059 grafts, made 4 to 9 years previously, were dead due to incompatibility. The 46-percent loss is not the final incompatibility value, as many surviving grafts already show the scion overgrowth symptom and will probably die.

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<sup>1/</sup> Duffield, J. W., and Wheat, J. G. Graft failures in Douglas-fir. J. Forest. 62: 185-186. 1964.

Early detection of incompatibility in new clones would be of great value to Douglas-fir seed orchardists. Unfortunately, D.I. clones go undetected for a number of years. During that time, the rootstocks become too large for grafting with replacement clones. This necessitates costly roguing of large stocks. Also, the newly planted stocks and later grafted scions are generally in a subordinate stand position in the developing orchard.

Mosse<sup>2/</sup> and Herrero,<sup>3/</sup> with plum and pear grafts, and Lapins,<sup>4/</sup> with apricot grafts, demonstrated that early tests for incompatibility are possible for some fruit trees. They detected anatomical symptoms in 2d-year grafts which could be used to make accurate estimates of incompatibility. Furthermore, the field performance of older grafts from the same clones was closely correlated with predictions based on 2d-year graft anatomy. Copes<sup>5/</sup> later discovered 2d-year symptoms in Douglas-fir grafts which could be used to accurately detect delayed incompatibility. This paper describes how orchardists can make use of this technique.

### Basis of Testing

To accurately estimate graft incompatibility, two factors must be considered: amount of E.I. mortality and amount of wound xylem caused by D.I. It is necessary, however, to separate E.I. losses from those caused by grafting technique. Any failure before a graft is 4 months old is considered a loss from faulty grafting. Graft failures between the 4th and 24th months are considered losses caused by E.I.

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<sup>2/</sup> Mosse, B. Further observations on growth and union structure of double-grafted pear on quince. J. Hort. Sci. 33: 186-193. 1958.

Mosse, B. Graft incompatibility in plums: Observations on a ten-year-old field trial. J. Hort. Sci. 35: 260-265. 1960.

<sup>3/</sup> Herrero, J. Studies of compatible and incompatible graft combinations with special reference to hardy fruit trees. J. Hort. Sci. 26: 186-237, illus. 1951.

<sup>4/</sup> Lapins, K. Some symptoms of stock-scion incompatibility of apricot varieties on peach seedling rootstocks. Can. J. Plant Sci. 39: 194-230.

<sup>5/</sup> Copes, Donald L. Graft incompatibility and union formation in Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco. 187 pp. 1967. (Unpublished Ph.D. thesis on file at Univ. Idaho, Moscow.)

The second factor, the amount of D.I., is less easy to describe since it is concerned with the development of abnormal woody tissues which appear in the xylem union at the start of the second growing season. It will be best, therefore, to consider (1) what the D.I. symptom is, (2) how the symptom develops, and (3) how this symptom can be used in a test for incompatibility. Delayed incompatibility in Douglas-fir is always associated with the formation of wound-xylem areas. The presence of wound xylem is a positive symptom of D.I.<sup>6/</sup> Wound-xylem areas are composed of living callus cells, disorganized tracheids, and, sometimes, engulfed areas of dead bark cells. When examined late in the second growing season, each wound area appears as a small island of abnormal tissue (fig. 1). Wound xylem caused by incompatibility is found in all union zones where stock and scion cambial tissues merge (fig. 2).

Second, at the start of the second growing season, delayed incompatible grafts have dead cambial tissues in each union area where the stock and scion cambiums join. Grafts which do not die of E.I. undergo at this time a natural regrafting of stock and scion tissues. Living cells of the bark form new callus tissue on both the scion and stock sides of the dead cambium and, after much cell division, form a bridge of living tissue between the stock and scion. A cambium later differentiates in each bridge area, and for the remainder of that growing season, relatively normal tracheids are formed. This regrafting process results in cell types and tissue structures that are similar to those occurring after man-made grafting (fig. 2). The formation of callus and the resulting bridge are part of the characteristic tissue which I have termed "wound-xylem areas."<sup>7/</sup>

The same pattern of cambial necrosis and natural regrafting occurs at the start of the third, fourth, and every following year until scion death (fig. 2). Death of D.I. grafts occurs after they fail to regraft. Regraft failure causes a girdling effect, and the scion develops the characteristic overgrowth described by Duffield and Wheat.<sup>8/</sup> When the roots starve from lack of photosynthates, or when the scion dies of desiccation after the connecting xylem becomes nonfunctional, the graft can be said to have died of D.I. Death may occur in the same year of regraft failure, or the graft may live as long as another 4 years. All grafts that form this type of wound-xylem area must be classified as a potential source of loss.

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<sup>6/</sup> See footnote 5.

<sup>7/</sup> See footnote 5.

<sup>8/</sup> See footnote 1.



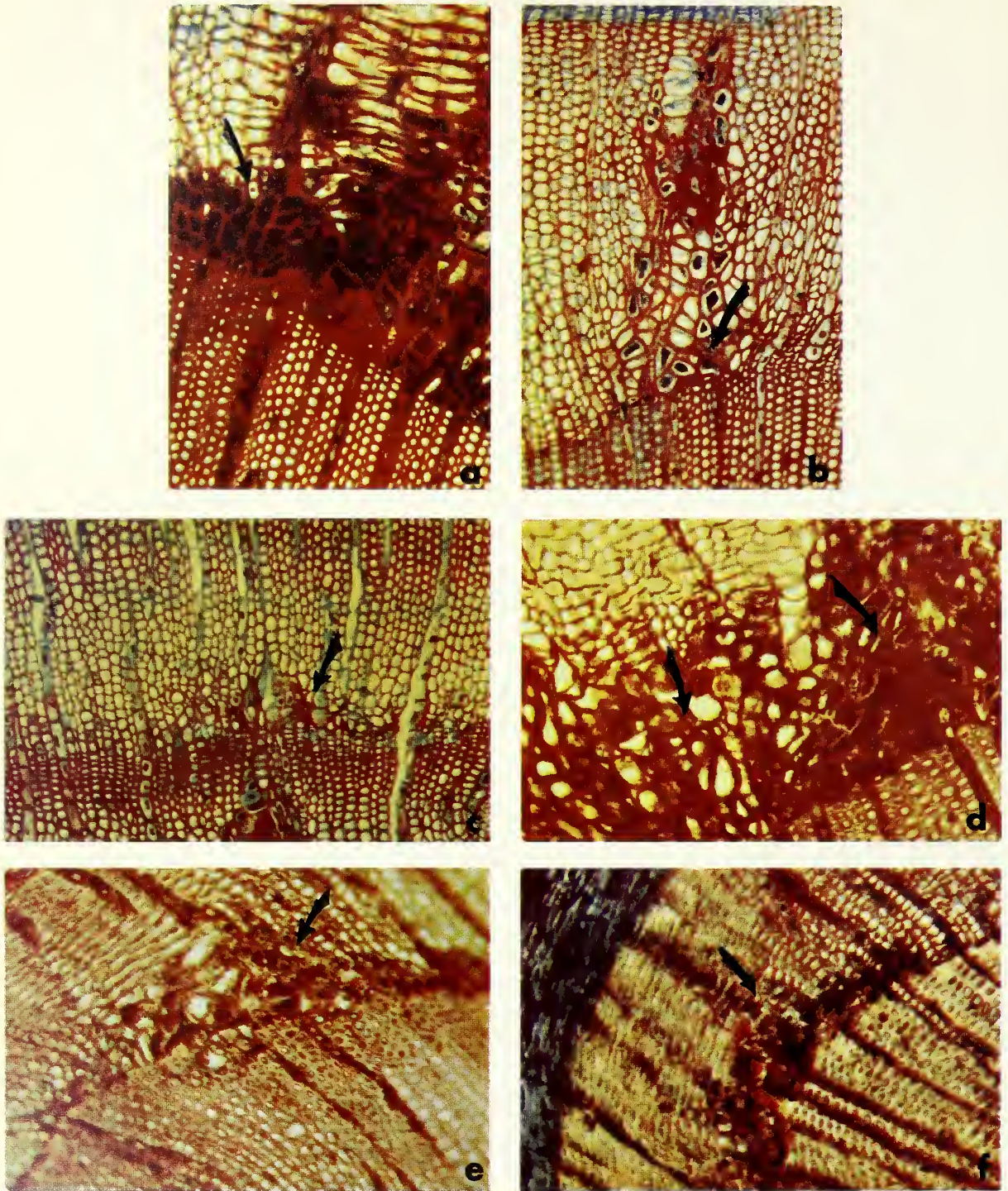
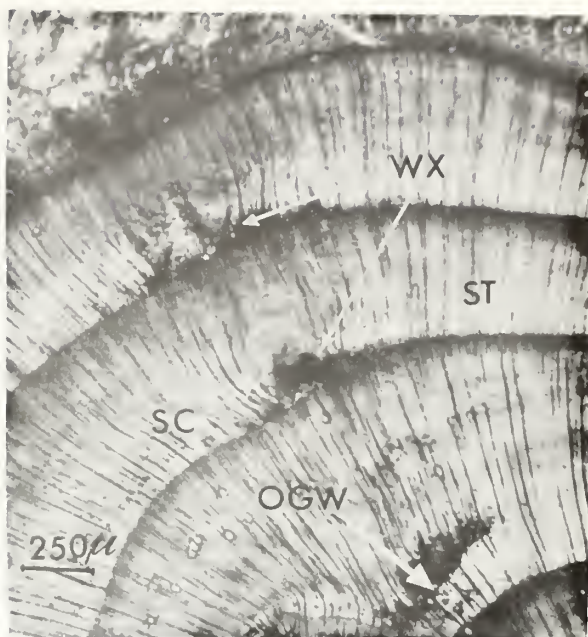


Figure 1.--Typical wound-xylem areas are shown in union zones of incompatible grafts at the start of the second season following grafting. The 1966 xylem is toward the bottom of each photograph, and 1967 xylem is toward the top. Sections shown in photos a, b, c, and f were stained with both safranin and fast green, whereas those in photos d and e were stained with safranin only. The arrows point to the wound areas.



*Figure 2.--Wound-xylem areas formed in one union zone of an incompatible graft after natural regrafting in the second and third growing seasons. Note the similarity between those areas and the original graft tissues formed as a result of man-caused grafting. (Wound xylem, WX; original graft wound, OGW; scion, SC; stock, ST.)*



Not all wound areas are necessarily symptoms of incompatibility. Frost damage and poor grafting technique can cause the formation of wound areas resembling those due to incompatibility, but they can be differentiated. First, frost damaged tissue is not found solely in the union zones, as are wound-xylem areas. Second, poor grafting technique can occasionally cause the formation of wound-xylem areas closely resembling those found in incompatible grafts, but such areas occur only where a physical disruption of the union exists. Also, other union zones within the same graft show no evidence of wound xylem, whereas a truly incompatible graft would show wound xylem in all areas where stock and scion cambiums were in contact.

### Requirements for an Early Test for Incompatibility

An early test for incompatibility will require the following steps:

1. Establishment of at least 10 grafts of each clone for testing; allow a few extras for technique failures.
2. Separation of grafting technique losses from E.I. losses.
3. Sectioning of graft unions into thin cross sections to determine the presence or absence of wound-xylem areas (D.I.).

Early testing of new clonal selections before they are grafted into the orchard will be of great value to orchardists.

Grafts for the incompatibility test can be made on either potted, nursery, or wildling stocks. It is possible that the most accurate screening for incompatibility will be obtained when graft techniques, time of grafting, stocks, and environmental conditions closely match those which are found in actual orchards. Since orchard grafting is done on many different stocks, it is desirable to test-graft new clones on as many stock genotypes as possible. No more than one graft of each clone should be made on a single stock, but it is possible to make efficient use of available stocks by grafting more than one clone on each stock. Two, three, or four grafts of a clone on the same stock will not tell the orchardist any more about the compatibility of that scion and stock combination than will a single graft. Grafts placed on branches are as accurate for test purposes as grafts placed on main leaders.

Collections made before growth starts in the 2d year will not show wound-xylem areas or tell the orchardist much concerning the D.I. of the clones being tested. The preferred collection time is either in late summer or fall after most diameter growth has ceased. Wound-xylem areas are checked more easily if they are surrounded by normal tissue.

Screening can also be done on grafts older than 2 years, but sectioning is difficult with the large unions of older grafts. Therefore, 2-year-old unions are preferred for screening.

### Screening Procedure for Individual Clones

The technical procedure for testing clones for incompatibility is as follows:

#### A. Estimating Early Incompatibility

1. For each clone, subtract the mortality losses through the first 4 months from the total number of grafts. The difference is the test base for estimates of incompatibility.
2. For each clone, record the number of grafts which died between 4th month and time of graft union collection. This value represents losses from early incompatibility.
3. To determine the percentage of early incompatibility for each clone, divide the value from step 2 by the test base obtained in step 1 and multiply the results by 100. The orchardist will have to decide which clones should be eliminated from testing owing to severe losses from E.I.



## B. Estimating Delayed Incompatibility

1. Collect unions of surviving grafts of each clone during the summer or fall of the 2d year. Sever the stem immediately above and below the union. Place the unions in vials or jars containing a 30- to 50-percent solution of isopropyl or ethyl alcohol. The grafts can be stored in this solution indefinitely. Allow the grafts to remain in the solution for at least 1 day before proceeding with step 2.
2. Remove the grafts from the solution immediately before cutting cross sections from the middle of the union. A razor blade or an extremely sharp grafting knife can be used for cutting. Cut the sections as thin as possible. Only one good section is needed from each graft, but several should be cut in case the first ones are too thick, badly cut, or poorly stained.
3. Place the cut sections for at least 10 seconds in a small dish containing 50-percent solution of ethyl or isopropyl alcohol. This will prevent the sections from drying out and prepare them for step 4. A pair of tweezers is a useful tool for moving sections in steps 3 to 8.
4. Move the sections into a dish of safranin.<sup>9/</sup> Allow the sections to remain in the stain for at least 1 minute (fig. 1e).
5. Wash the stained section in a dish containing 95-percent solution of ethyl or isopropyl alcohol. Washing and destaining normally requires only 10 to 20 seconds. No overstaining with safranin in step 4 is possible because excess stain can be removed by adding a few drops of HCl acid to this 95-percent alcohol solution.
6. Place the sections, one at a time, into a dish of fast green<sup>10/</sup> and leave for 2 to 10 seconds.
7. Place the stained section in a dish of 95-percent solution of ethyl or isopropyl alcohol until no more stain washes off. This takes only 5 to 10 seconds. Woody tissues stain light red and the bark a dark purple or blue (fig. 1f).

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<sup>9/</sup> Safranin O staining is done with one part safranin O to 99 parts alcohol (50-percent solution).

<sup>10/</sup> Fast green staining is done in a saturated solution of fast green in 95-percent alcohol solution. Make the staining solution at least 1 day before use.

8. Place the stained sections on a microscope slide and add enough 95-percent alcohol solution to prevent drying of the sections while the slide is being examined. A cover glass should be lowered carefully on the section. If it is not lowered gradually, there will be air bubbles trapped between the slide and cover glass, and this makes observation of wound-xylem areas difficult.
9. Examine the slide under a microscope. Look for wound-xylem areas in each union zone where a cambium connected the scion and the stock at the start of the 2d year after grafting. Incompatible grafts develop a wound in each zone. Determine how many grafts of each clone formed wound-xylem areas.
10. For each clone, add the number of grafts which died of early incompatibility (A-2 value) to the number of grafts which had wound xylem (B-9 value). Divide the sum by the test base number of grafts living after 4 months (A-1 value) and multiply the result by 100. The resulting value is the percentage of incompatibility shown by the tested clone.
11. At this stage, the orchardist should again exclude clones which are too incompatible to warrant grafting.

### Equipment and Supplies

1. Ninety-five-percent solution of ethyl or isopropyl alcohol.
2. Vials or small jars for storing the grafts and alcohol fixative (corks or covers are needed).
3. A sharp knife or razor blades.
4. Six petri dishes or other small dishes.
5. Microscope capable of 100X magnification (a low-power dissecting scope capable of 10-30X magnification is a very helpful supplement but cannot replace the larger microscope).
6. Fast green stain.
7. Safranin O stain.
8. Tweezers.
9. Microscope slides and coverslips.